INFLUENCE OF PHOSPHORUS AND NITRATES ON THE SPECIES DEVELOPMENT OF *LEMNA MINOR* L.

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Abstract
In this paper we wanted to determine the influence of phosphorus and nitrates in the development of *Lemna minor* L. To achieve this purpose, eight different growth variants were proposed, starting from the Hoagland culture medium, where the concentration of these two compounds varied. The growth rate in the eight experimental variants, ranges between 150% - 325%, with a minimum in the control sample and a maximum at the highest concentration of phosphorus in water.

Introduction
Duckweed (*Lemna minor* L.) is a widespread aquatic plant located in lakes, rivers, ponds and other bodies of water [1]. *L. minor* L. belongs to the family Araceae [2], being an aquatic (submerged) plant with free (floating) root, consisting of small leaves. The size of the leaves is between 0.5-1.0 cm and the root length is about 3.0-4.0 cm.
This plant has been reported in numerous phytoremediation studies to remove pesticides and heavy metals from water [3, 4] and it is therefore important to determine the optimum conditions for plant growth in order to ensure the plant material needed for these studies.
Aquatic plants absorb the organic and inorganic nutrients dissolved in the water column and these can be exhausted to the extent that the growth of aquatic plants is limited by nutrients [5]. Nitrogen compounds, including nitrates, along with phosphorus, are among the most important nutrients in crops, and are limiting factors in ontogenetic plant development.
Low levels of phosphate limit plant growth in both land and aquatic systems [6], however, excess nitrogen compounds and excess phosphorus in an aquatic environment stimulate excessive production of algae and phytoplankton, which leads to eutrophication [7, 8], therefore it is imperative to know and adapt the amounts of nutrients used in the growth of aquatic plants.

Experimental
Vegetal material and plant development variants
The plants were collected from a natural environment between March and April and were transferred to the laboratory, where they was thoroughly washed with distilled water to remove impurities, and then introduced into the Hoagland's culture medium, described by Cowgill & Milazzo in 1989 [9]. The Hoagland's culture medium is described in tables 1 and 2.

Table 1. Hoagland's culture medium preparation

Composition	**Preparation stock solution**	**Quantity used**
1. $MgSO_4 \cdot 7H_2O$	24.6 g/100 mL	1.0 ml/L
2. $Ca(NO_3)_2 \cdot 4H_2O$	23.6 g/100 mL	2.3 ml/L
3. KH_2PO_4	13.6 g/100 mL	0.5 ml/L
4. KNO_3	10.1 g/100 mL	2.5 ml/L
5. Micronutrients	See below (table 2)	0.5 ml/L

Table 2. Preparation of micronutrients solution

H_3BO_3	2.86 g/L
$MnCl_2 \cdot 4H_2O$	1.82 g/L
$ZnSO_4 \cdot 7H_2O$	0.22 g/L
$Na_2MoO_4 \cdot 2H_2O$	0.09 g/L
$CuSO_4 \cdot 5H_2O$	0.09 g/L

Experiments were performed on eight lot, using different reagent quantities (to determine the influence of nitrates and phosphorus on plant growth), presented in the Hoagland's culture medium, as follows: Lot 1: culture medium in which the phosphorus concentration was varied by the addition of 0.25 mL of dihydrogen phosphate; Lot 2: culture medium in which the phosphorus concentration was varied by the addition of 0.75 mL of dihydrogen phosphate; Lot 3: culture medium in which the phosphorus concentration was varied by the addition of 1.00 mL of dihydrogen phosphate; Lot 4: culture medium in which the nitrate concentration was varied by the addition of 1.20 mL of calcium nitrate and 1.25 mL of potassium nitrate; Lot 5: culture medium in which the nitrate concentration was varied by the addition of 3.5 mL of calcium nitrate and 3.75 mL of potassium nitrate; Lot 6: culture medium in which the nitrate concentration was varied by the addition of 4.6 mL of calcium nitrate and 5 mL of potassium nitrate; Lot 7: Hoagland's culture medium, according to tables 1 and 2; Lot 8: control sample. For the control sample, water from the natural environment (pond water) where the plants was harvested, was used.

Experimental conditions

Approximately 4 grams of plants were placed in plastic containers containing one of the growth variants shown above of size Lxlxh = 20x16x6 cm. The experimental duration was 7 days at a temperature between 19 °-25 ° C and a day / night cycle of 15/9. The culture media described in the above lots, was renewed on day 4 to support the growth rate and to compensate the deficiency of water compounds, vital compounds in plant growth. The plants were placed in the laboratory at the window leve, exposed directly to sunlight.

Calculating the growth rate of plants

Growth rate is the degree of growth and development of plants, over a period of time. The growth rate is calculated according to the initial plant weight used and the weight of the plants at the end of the experiments. The resulting value is expressed as a percentage (%).

$$Growth\ rate(\%) = \frac{\text{final biomass} - \text{initial biomass}}{\text{initial biomass}} x100$$

Results and discussion

All experiments data, presented in the present paper represent the average of three samples.

In the following tables, the growth and development of plants at different concentrations of macro-nutrients and in particular of phosphorus and nitrogen compounds was pursued.

Interpreting the tables below, it can be observed the decisive influence of phosphorus on the rate of plant growth. The baseline recipe shows a 275% plant growth in the presence of 16 mg/L phosphorus and 369 mg/L nitrated in water (lot 7). Comparing lots 1, 2, 3 and 7, in which the phosphorus concentration in water varied (the rest of the elements remained approximately unchanged), the highest increase was found in lot 3 (at the highest concentration of phosphorus: 26.0 mg/L), the increase being 325%.

For lots 4, 5, 6 and 7 in which the nitrate concentration varied (the rest of the elements remained approximately unchanged), it can be said that their influence in the water is quite small, the growth rates being in the range of 200-275% with a maximum increase in the nitrate concentration in water of 369 mg/L. The lowest values of the growth rate (200% - lot 5 and 225% - lot 6) are observed at 644 mg/L and 846 mg/L nitrate respectively, whereby high concentrations of nitrates inhibit ontogenetic development of plants.

The control sample (lot 8), differs from the rest of the growth variants by the lowest proliferation of plants, with a growth rate of only 150%, demonstrating the influence and importance of micro and macro-nutrients and, in particular, of phosphorus compounds and nitrogen for an accelerated development of aquatic plants, especially *Lemna minor* L.

Table 1. Modified culture medium (with 0.25 mL dihydrogen phosphate) (lot 1)

pH	Phosphorus (mg/L)	Ammonium (mg/L)	Nitrates (mg/L)	Nitrites (mg/L)	Initial weight (g)	Final weight (g)
8,2	8,00	0,247	412	0,096	4	11
Growth rate (%)=175						

Table 2. Modified culture medium (with 0.75 mL of dihydrogen phosphate) (lot 2)

pH	Phosphorus (mg/L)	Ammonium (mg/L)	Nitrates (mg/L)	Nitrites (mg/L)	Initial weight (g)	Final weight (g)
7,9	22,0	0,241	388	0,080	4	15
Growth rate (%)= 275						

Table 3. Modified culture medium (with 1 mL dihydrogen phosphate) (lot 3)

pH	Phosphorus (mg/L)	Ammonium (mg/L)	Nitrates (mg/L)	Nitrites (mg/L)	Initial weight (g)	Final weight (g)
7,8	26,0	0,223	370	0,094	4	17
Growth rate (%)= 325						

Regarding the influence of pH on plant growth (in the above mentioned variants, a pH between 6.6-8.8 was determined), this is not a limiting factor according to the literature, in which many authors reported that the duckweed species resist at a wide range of pH, within the range 3.5-10 [4].

Table 4. Modified culture medium (with 1.2 mL of calcium nitrate and 1.25 mL of potassium nitrate) (lot 4)

pH	Phosphorus (mg/L)	Ammonium (mg/L)	Nitrates (mg/L)	Nitrites (mg/L)	Initial weight (g)	Final weight (g)
8,1	14,0	0,226	203	0,065	4	14
Growth rate (%)=250						

Table 5. Modified culture medium (with 3.5 mL of calcium nitrate and 3.75 mL of potassium nitrate) (lot 5)

pH	Phosphorus (mg/L)	Ammonium (mg/L)	Nitrates (mg/L)	Nitrites (mg/L)	Initial weight (g)	Final weight (g)
8,0	15,0	0,201	644	0,262	4	12
Growth rate (%)=200						

Table 6. Modified culture medium (with 4.6 mL of calcium nitrate and 5 mL of potassium nitrate) (lot 6)

pH	Phosphorus (mg/L)	Ammonium (mg/L)	Nitrates (mg/L)	Nitrites (mg/L)	Initial weight (g)	Final weight (g)
7,9	16,0	0,196	846	0,116	4	13
Growth rate (%)= 225						

Table 7. Hoagland's culture medium (lot 7)

pH	Phosphorus (mg/L)	Ammonium (mg/L)	Nitrates (mg/L)	Nitrites (mg/L)	Initial weight (g)	Final weight (g)
6,6	16,0	<0,025	369	<0,024	4	15
Growth rate (%)= 275						

Table 8. Control sample - pond water (lot 8)

pH	Phosphorus (mg/L)	Ammonium (mg/L)	Nitrates (mg/L)	Nitrites (mg/L)	Initial weight (g)	Final weight (g)
8,8	<0,017	<0,025	<0,074	<0,024	4	10
Growth rate (%)= 150						

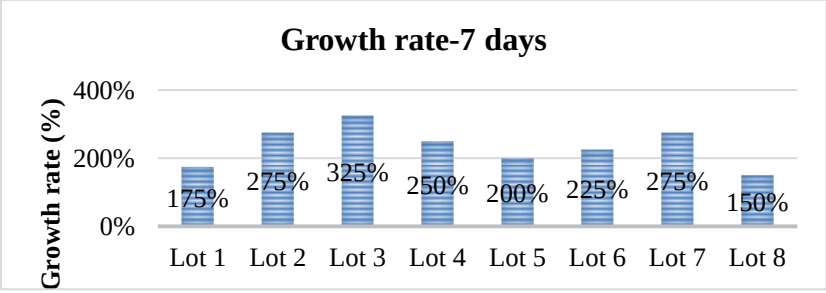

Figure 1. Synthetic presentation of growth rates after 7 days

Figure 1 shows the growth of the plant *Lemna minor* L., from which we can see that the greatest development of the plants we have in the lot number 3, and the lowest in the lot with number 8, the latter being control sample.

For the highest growth rates, ranging from 275% to 325%, the optimal conditions for growth/development of *Lemna minor* L. in terms of nutrient content in water are defined (Table 9).

Table 9. Optimum conditions for the development of the *Lemna minor* L. plant, in terms of nutrient content

pH	Phosphorus (mg/L)	Ammonium (mg/L)	Nitrates (mg/L)	Nitrites (mg/L)
6,6 - 7,9	16,0 -26,0	0,025- 0,241	approx. 369	0,024 -0,094

Conclusion

In experiments on the variation of the phosphorus concentration in water, it can be seen that the highest proliferation of the plant, of 325%, is in the presence of phosphorus concentration in the water of 26.0 mg/L.

In experiments in which nitrate concentration varied, we can mention that the maximum growth rate (275%) is observed at water nitrate concentrations of 369 mg/L.

High concentrations of phosphorus lead to plant proliferation, while high concentrations of nitrates inhibit their growth.

In the presented above, it is found that the greatest influence on the growth rate of *Lemna minor* L. is phosphorus, followed by nitrogen compounds, from where it results, in studies in which multiplication of the plant is desired, this aspect must be taken into account.

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